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*J Immunol* 2006; 176:111-117; doi: 10.4049/jimmunol.176.1.111
http://www.jimmunol.org/content/176/1/111

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*The Journal of Immunology* is published twice each month by The American Association of Immunologists, Inc., 1451 Rockville Pike, Suite 650, Rockville, MD 20852
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Print ISSN: 0022-1767 Online ISSN: 1550-6606.
α-Lipoic Acid Inhibits Inflammatory Bone Resorption by Suppressing Prostaglandin E₂ Synthesis

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α-Lipoic acid (LA) has been intensely investigated as a therapeutic agent for several pathological conditions, including diabetic polyneuropathy. In the present study, we examined the effects of LA on osteoclastic bone loss associated with inflammation. LA significantly inhibited IL-1-induced osteoclast formation in cocultures of mouse osteoblasts and bone marrow cells, but LA had only a marginal effect on osteoclastogenesis from bone marrow macrophages induced by receptor activator of NF-κB ligand (RANKL). LA inhibited both the sustained up-regulation of RANKL expression and the production of PGE₂ induced by IL-1 in osteoblasts. In addition, treatment with either prostaglandin E₂ (PGE₂) or RANKL rescued IL-1-induced osteoclast formation inhibited by LA or NS398, a specific cyclooxygenase-2 (COX-2) inhibitor, in cocultures. LA blocked IL-1-induced PGE₂ production even in the presence of arachidonic acid, without affecting the expression of COX-2 and membrane-bound PGE₂ synthase. Dihydrolipoic acid (the reduced form of LA), but not LA, attenuated recombinant COX-2 activity in vitro. LA also inhibited osteoclast formation and bone loss induced by IL-1 and LPS in mice. Our results suggest that the reduced form of LA inhibits COX-2 activity, PGE₂ production, and sustained RANKL expression, thereby inhibiting osteoclast formation and bone loss in inflammatory conditions. The Journal of Immunology, 2006, 176: 111–117.

Bone remodeling depends on maintaining a delicate balance between bone resorption by osteoclasts and bone formation by osteoblasts. Tipping this balance in favor of osteoclasts leads to bone destruction in pathological bone diseases such as periodontitis, osteoporosis, and arthritis (1, 2). Osteoclasts are derived from the monocyte-macrophage lineage under the presence of both the TNF-related factor known as receptor activator of NF-κB ligand (RANKL) and M-CSF, which are produced by osteoblasts or stromal cells (3–6). Osteoprotegerin (OPG), a soluble decoy receptor for RANKL, is produced by osteoblasts and inhibits osteoclast formation by blocking RANKL binding to RANK (7, 8). A variety of factors, including parathyroid hormones, 1,25-dihydroxyvitamin D₃ (Vit D₃), and proinflammatory cytokines can increase osteoclast formation via up-regulating the expression of RANKL and/or down-regulating OPG in osteoblasts and stromal cells (9).

IL-1 is a proinflammatory cytokine that is a potent stimulator of bone resorption and inhibitor of bone formation. A variety of cells in the bone microenvironment, including monocyte/macrophages, osteoblasts, and osteoclasts, can synthesize IL-1 (10, 11). Although IL-1 has been known to stimulate osteoclast formation by increasing the production of prostaglandin E₂ (PGE₂) and the ratio of RANKL:OPG in osteoblasts and stromal cells (9, 12), the relationship of the two factors in IL-1-induced osteoclast formation has not been clearly elucidated.

α-Lipoic acid (LA) is a naturally occurring, short-chain fatty acid containing two sulfur molecules. It is an essential cofactor of mitochondrial respiratory enzymes (pyruvate dehydrogenase and α-keto-glutarate dehydrogenase) (13, 14). LA is both water and fat soluble and, therefore, is distributed in both the cellular membranes and the cytosol in plants and animals (15). LA is taken up and reduced by cells to dihydrolipoic acid (DHLA), which can be released into the extracellular medium (16, 17). LA and DHLA can serve as powerful antioxidants through several mechanisms, including scavenging of free radicals, chelation of metal ions, and regeneration of endogenous and exogenous antioxidants, such as ubiquinon, vitamins C and E, and glutathione (13, 18–20). Pharmacologically, LA has been investigated as a therapeutic agent in the treatment or prevention of several pathological conditions. Intravenous infusion of LA reduces symptoms of diabetic peripheral neuropathy in human (21). Dietary LA supplementation prevents hypertension, insulin resistance, and aorta superoxide production in a rat model of hypertension induced by chronic glucose feeding (22). LA has recently been shown to exert potent antiobesity effects by suppressing hypothalamic AMP-activated protein kinase activity (23). The powerful antioxidant activity of LA has also been implicated in the prevention or alleviation of neurodegeneration, ischemia–reperfusion injury, AIDS, and hepatic diseases (13, 24, 25).

Despite rigorous studies, little is known about the effects of LA on osteoclastic bone loss. Therefore, we examined the effects and potential targets of LA on osteoclast formation associated with inflammation. We show in this study that LA prevents osteoclast formation induced by IL-1 in vitro and in vivo by inhibiting PGE₂ synthesis.
Materials and Methods

**Reagents**

α-MEM and FBS were purchased from Invitrogen Life Technologies. Recombinant IL-1α, RANKL, and M-CSF were obtained from PeproTech. Arachidonic acid and NS398 were purchased from Calbiochem. LPS (Escherichia coli 0111:B4), N-acetyl-L-cysteine (NAC), and glutathione (GSH) were purchased from Sigma-Aldrich.

**Cell cultures**

Primary osteoblasts were prepared from calvaria of newborn ICR mice as previously described (26). Bone marrow cells were isolated from femurs and tibias of 5- to 7-wk-old ICR mice. Mouse bone marrow cells were plated on 10-cm culture dishes and incubated overnight. Nonadherent bone marrow cells were transferred to bacterial culture dishes and cultured in the presence of M-CSF (50 ng/ml) for 3 days. Adherent cells were used as bone marrow macrophage (BMM) after washing out the nonadherent cells.

**In vitro osteoclast cultures**

For osteoclast formation assays, primary osteoblasts (2.5 × 10^5 cells) and bone marrow cells (3 × 10^5 cells) were cocultured for 7 days in 1 ml of α-MEM containing 10% FBS and IL-1α (10 ng/ml) in 48-well culture plate. Cells positively stained for tartrate-resistant acid phosphatase (TRAP) and containing more than three nuclei were counted as osteoclasts. For some experiments, osteoclasts were generated from BMMs cultured for 4 days with M-CSF (30 ng/ml) and RANKL (100 ng/ml) in the presence of the absence of LA or DHLA.

**Measurement of PGE2 content**

Primary osteoblasts (3 × 10^4 cells) were cultured for 24 h with or without IL-1α (10 ng/ml) in α-MEM containing 10% FBS in 12-well culture plate. Some cultures were also treated with LA, DHLA, or GSH. The concentration of PGE2, in the culture medium was measured using an enzyme immunoassay (ElA; Amesham Biosciences). The Ab had the following cross-reactivities when calculated by the bound/free ratios with several eicosanoids: PGE2, 100%; PGE1, 25%; PGF2α, 0.04%; and 6-keto-PGF1α, <0.1%.

**Western blot analysis**

Western blotting analyses were performed as previously described (26). Briefly, cells were lysed in a buffer containing 20 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, and protease and phosphatase inhibitors. Cell lysates (50 μg/lane) were subjected to SDS-PAGE, then transferred to polyvinylidene difluoride membranes. The blots were probed with each primary Ab in a 1/1000 dilution. The primary Abs used in this study were anti-cytosolic phospholipase A2 (New England Biolabs), anti-cyclooxygenase-2 (New England Biolabs), and anti-membrane-bound PGE synthase-1 (mPGES-1) (Cayman Chemical). Blots were finally developed by using HRP-conjugated secondary Abs and visualized using ECL (Amersham Biosciences).

**In vitro recombinant cyclooxygenase-2 (COX-2) activity**

Human recombinant COX-2 activity was measured using a COX activity assay kit following the manufacturer’s instructions (Cayman Chemical). In brief, 30 U of human rCOX-2 were incubated for 15 min with or without LA, DHLA, or GSH in the presence of heme, then incubated for an additional 5 min with colorimetric substrate (N,N,N′,N′-tetramethyl-p-phenylenediamine) and arachidonic acid solution. The peroxidase activity of COX was assayed colorimetrically by monitoring the appearance of oxidized N,N,N′,N′-tetramethyl-p-phenylenediamine at 590 nm.

**Semi quantitative RT-PCR analysis**

Total RNA was extracted from primary osteoblasts with TRIzol reagent, and cDNA was synthesized from 2 μg of total RNA by reverse transcription (Script Super II Preaplication System; Invitrogen Life Technologies). Semi quantitative RT-PCR was performed on serial dilutions of each cDNA. The following primers were used: mouse RANKL: forward, 5'-CAGTTTGGACACTGCAG-3'; and reverse, 5'-ACGAGGGGACTGTTCGC-3'; mouse OPG: forward, 5'-CACTCTTATACAGGACAGCT-3'; and reverse, 5'-CTCCGCCATCTTTGGTCTC-3'; and mouse GAPDH: forward, 5'-ACCACACCCGCTCACTACA-3'; and reverse, 5'-TCCACCCGCTGCTGCTGTA-3'. PCRs were conducted for 21 cycles (for mouse GAPDH) or 26 cycles (for mouse RANKL and OPG) at 94°C for 30 s, 52°C for 30 s, and 72°C for 1 min. The amplified cDNA fragments were run on 1.5% agarose gel, stained with ethidium bromide, and detected under UV light.

**Statistical analysis**

All quantitative data are presented as the mean ± SD. Each experiment was performed four or five times, and results from one representative experiment are shown. Statistical differences were analyzed by Student’s t test. A value of p < 0.05 was considered statistically significant.

**Results**

**LA inhibits osteoclast formation by IL-1 in cocultures**

We first examined the effect of LA on IL-1-induced osteoclast formation in cocultures of primary osteoblasts and bone marrow cells. Treatment with IL-1 for 7 days stimulated TRAP-positive

**In vivo experiments**

To study the effects of LA on IL-1-induced osteoclast formation in vivo, mice were implanted over the calvarial bones with collagen sponge treated with vehicle or IL-1 (1 μg), then received injections i.p. with vehicle (DMSO) or LA (25 mg/kg body weight) twice a day beginning on day 0. The mice were killed 7 days after the implantation, and intact calvariae were fixed in 4% paraformaldehyde and stained for TRAP. Bone loss induced by LPS administration was performed using previously described methods (12) with a slight modification. Mice received injections i.p. with LPS (5 mg/kg body weight) on days 0 and 4 and also received injections i.p. with vehicle (DMSO) or LA (25 mg/kg) twice a day beginning on day 0. The femurs were collected on day 8 after the first injection. The left femur of each animal was scanned with a high resolution microCT (SkyScan 1072 microCT system; SkyScan). The microCT system used an x-ray charge-coupled device camera with a cooled 1024 × 1024-pixel, 12-bit sensor. The femurs were fixed in 4% paraformaldehyde, decalified with 12% EDTA, and then embedded in paraffin. Histological sections were prepared and stained for TRAP. The trabecular bone volume density (bone volume/tissue volume) and the mean number of osteoclasts in each millimeter of the trabecular bone surface (millimeters) were determined in femoral cancellous bone using microCT and TRAP-stained sections, respectively. All animal experiments were reviewed and approved by the Seoul National University School of Dentistry animal care committee.
osteoclast formation in the cocultures. LA significantly inhibited osteoclast formation induced by IL-1 in a dose-dependent manner (Fig. 1A). Almost complete inhibition, even in TRAP-positive mononucleated cells, was observed at 20 μM LA (Fig. 1, A and D, *upper panels*). To determine at which stage LA inhibits osteoclast formation, the cocultures were incubated with LA for 0–7, 0–3, or 3–7 days (Fig. 1B). The incubation of LA during the early (0–3 days) or late (3–7 days) period significantly inhibited osteoclast formation compared with the control. Thus, LA affected a molecule required for osteoclastogenesis in both periods. The inhibitory effect of LA was not due to cytotoxicity or reduced cell growth. Incubation of cocultures or BMM cultures with LA (10–50 μM) for 48 h did not affect cell proliferation evaluated using the MTT assay (data not shown). To further characterize the effect of LA on osteoclast formation, osteoclasts were differentiated from BMM cultures in the presence of M-CSF and RANKL. The addition of LA had a minimal effect only at high concentrations on osteoclast formation from BMM cultures, unlike the potent effect observed in cocultures (Fig. 1, C and D, *lower panels*).

**LA interferes with the sustained expression of RANKL induced by IL-1**

IL-1 has been shown to promote osteoclastogenesis from osteoclast progenitors by regulating the expression of RANKL and OPG in osteoblasts or stromal cells (27). Thus, we next analyzed the effects of LA on the expression levels of RANKL and OPG mRNA in osteoblasts by RT-PCR analysis. Treatment of osteoblasts with IL-1 increased the expression level of RANKL mRNA within 1 h, and this effect was sustained for 72 h (Fig. 2A). LA dose-dependently inhibited IL-1-induced RANKL expression at 72 h. However, LA did not affect the initial increase in RANKL expression by IL-1 (Fig. 2A). IL-1 reduced the OPG mRNA level at 72 h, and the presence of LA slightly attenuated this effect of IL-1. These results indicate that the inhibitory effect of LA on IL-1-induced osteoclastogenesis was mainly due to suppression of RANKL expression in osteoblasts. Therefore, we next examined whether the effect of LA could be overcome by supplying RANKL. The exogenous addition of RANKL significantly increased IL-1-induced osteoclast formation and recovered the inhibitory effect of LA on IL-1-induced osteoclast formation in cocultures (Fig. 2B).

**PGE2 rescues IL-1-induced osteoclast formation inhibited by LA**

In our preliminary results, treatment with Vit D (10 nM) or Vit D and PGE2 (100 nM) induced osteoclast formation in cocultures, and LA (20 μM) inhibited Vit D-induced, but not Vit D- and PGE2-induced, osteoclast formation (data not shown). PGE2 in bone is produced mainly by osteoblasts and acts as a mediator of osteoclast formation in response to several cytokines, including IL-1 (12, 28, 29). In addition, PGE2 increases RANKL expression in osteoblasts and stromal cells (24). Thus, we examined whether LA affected PGE2 synthesis induced by IL-1. We measured the PGE2 concentration in the culture medium of osteoblasts incubated with IL-1 in the presence or the absence of LA. The addition of IL-1 to osteoblasts markedly induced PGE2 production, and treatment with LA inhibited both basal and IL-1-induced PGE2 production in a dose-dependent manner (Fig. 3A). The addition of PGE2 (100 nM) to the cocultures completely recovered IL-1-induced osteoclast formation in the presence of LA (Fig. 3, B and C). These results imply that LA inhibited IL-1-induced PGE2 production in osteoblasts and thereby prevented osteoclast formation in the cocultures. The inhibition of IL-1 induction of RANKL mRNA by LA was blocked by addition of PGE2 (Fig. 3D). PGE2 (100 nM) alone slightly increased RANKL expression, and this effect was not affected by treatment with LA (Fig. 3D).
PGE₂ is required for sustained expression of RANKL by IL-1

The inhibition of IL-1-induced osteoclast formation by LA was reversed by addition of either RANKL (Fig. 2B) or PGE₂ (Fig. 3, B and C) to the cocultures. In addition, PGE₂ restored IL-1-induced RANKL expression inhibited by LA in osteoblasts (Fig. 3D). These results suggest that PGE₂ production induced by IL-1 is involved in RANKL expression in osteoblasts. To clarify the relationship between PGE₂ and RANKL expression, we used NS398 (1 μM), a specific inhibitor of COX-2. Similar to the results with LA, NS398 inhibited IL-1-induced RANKL expression at 72 h, but not at 1 or 3 h (Fig. 4A). Treatment with NS398 slightly inhibited the down-regulation of OPG expression by IL-1 at 72 h. In line with the inhibitory effect on RANKL induction, NS398 blocked osteoclast generation from IL-1-treated cocultures (Fig. 4B). Furthermore, the addition of RANKL or PGE₂ rescued IL-1-induced osteoclast formation blocked by NS398 in the cocultures (Fig. 4B).
**DHLA prevents IL-1-induced osteoclast formation**

We next examined the effects of DHLA, the reduced form of LA, on IL-1-induced osteoclast formation. IL-1 significantly induced the production of PGE2 and RANKL mRNA (Fig. 5, A and B). Like LA, DHLA dose-dependently inhibited not only the production of PGE2 (Fig. 5A), but also the elevation of RANKL mRNA expression (Fig. 5B) induced by IL-1 in osteoblasts. The addition of PGE2 rescued IL-1-induced expression of RANKL in LA-treated osteoblasts (Fig. 5B). DHLA also abolished IL-1-induced osteoclast formation in the cocultures, which was reversed by the addition of PGE2 (Fig. 5C). Furthermore, DHLA, except at the highest concentration, had no significant effect on osteoclast formation from BMMs treated with M-CSF and RANKL (Fig. 5D).

**LA decreases PGE2 production by inhibiting COX-2 activity**

We then examined how LA inhibited IL-1-induced PGE2 production. LA inhibited IL-1-induced PGE2 production in osteoblasts even in the presence of arachidonic acid (5 μM; Fig. 6A). This result implies that LA inhibits the step required for the conversion of arachidonic acid to PGE2. Previous reports have indicated that the production of PGE2 in response to IL-1 and LPS is dependent on the elevation of COX-2 and mPGES-1 expression (12, 29). In our study, treatment of osteoblasts with IL-1 for 24 h stimulated the protein expression of cytosolic phospholipase A2 (cPLA2), COX-2, and mPGES-1 (Fig. 6B). LA did not affect basal and IL-1-induced protein levels (Fig. 6B). We then checked COX-2 activity in vitro. DHLA and GSH, but not LA, decreased the peroxidase activity of human rCOX-2 (Fig. 6C). GSH and NAC had a weak effect only at high concentrations on IL-1-induced PGE2 production in osteoblasts (Fig. 6D). Taken together, these results suggest that LA enters the cells, is reduced to DHLA, and suppresses IL-1-induced PGE2 production by, at least in part, inhibiting COX-2 activity.

**LA prevents inflammation-induced bone destruction**

To examine the effects of LA on IL-1-induced osteoclast formation in vivo, we implanted a collagen sponge treated with or without IL-1 over the calvarial bones of mice. IL-1 significantly increased the number of osteoclasts and the resorbed area in calvarial bone, and this effect was inhibited by daily injection of LA (Fig. 7A). The same parameters were not appreciably changed in the presence of LA alone.

LPS, a major constituent of Gram-negative bacteria, has been reported to induce osteoclast formation in bone marrow cultures, and the administration of LPS stimulates osteoclastic bone resorption in part through elevation of PGE2 production in vivo (12, 30). To determine the influence of LA on bone loss induced by LPS administration, mice received injections with LPS with or without LA. The microCT analysis showed that LPS injection caused a marked bone loss, especially in the distal metaphysis of the femur. The loss of cancellous bone induced by LPS was significantly inhibited by daily injection of LA (Fig. 7, B and D). To determine the inhibitory effect of LA on LPS-induced bone loss, histological sections of distal femoral metaphysis were prepared and stained for TRAP. The injection of LPS significantly increased the number of osteoclasts in trabecular bone. In contrast, the number of osteoclasts in...
trabecular bone treated with LPS and LA was similar to that in the control (Fig. 7, C and D). These results imply that the inhibitory effect of LA on IL-1- or LPS-induced bone loss is due to the reduced number of osteoclasts.

Discussion
Production of IL-1 is involved in osteoporosis induced by both estrogen deficiency and inflammation. Mice lacking the type I IL-1R are resistant to bone loss after ovariectomy (31). Blocking the effects of IL-1 with IL-1R antagonists, anti-IL-1 mAbs, or soluble IL-1 type II receptors significantly reduces bone erosions and cartilage degradation in animal models of rheumatoid arthritis (32). In this study the addition of LA prevented osteoclast differentiation from cocultures by IL-1, but not from BMMs treated with M-CSF and RANKL (Fig. 1). LA significantly inhibited sustained RANKL expression in osteoblasts, without affecting its initial elevation by IL-1, and the exogenous addition of RANKL to the cocultures rescued the inhibitory effects of LA on osteoclast formation (Fig. 2). These results suggest that LA inhibited IL-1-induced osteoclast formation in the cocultures by inhibiting RANKL expression in osteoblasts, and that a factor required for maintenance of the up-regulated RANKL expression level might have been induced by IL-1.

Previous reports have shown that several proresorptive factors, including IL-1 (12, 28), TNF-α (28), LPS (29), and parathyroid hormone (33), stimulate osteoclast formation through PGE2-dependent mechanisms. We found that LA completely inhibited PGE2 production by IL-1 in osteoblasts, and the exogenous addition of PGE2 rescued not only RANKL expression in osteoblasts, but also osteoclast formation in cocultures, both suppressed by LA (Fig. 3). Using NS398, we also found that PGE2 production was required for the maintenance of RANKL expression by IL-1 (Fig. 4). These results indicate that LA decreases RANKL expression by inhibiting PGE2 synthesis in osteoblasts, thereby preventing osteoclast formation in the cocultures treated with IL-1.

PGE2 synthesis is regulated by three metabolic steps: the release of arachidonic acid from phospholipids, the conversion to PGH2, and the synthesis of PGE2 by PLA2, COX, and PGES (12). It was recently reported that cPLA2 is expressed in mouse osteoblasts and is the key enzyme in PGE2 synthesis in response to IL-1 and LPS (12). The two COX enzymes are encoded by separate genes and differentially expressed. Although constitutive COX (COX-1) and inducible COX (COX-2) are both expressed in mouse osteoblasts, COX-2 is largely responsible for PGE2 synthesis in osteoblasts after stimulation by several agonists (29, 33). At least three distinct PGES isoforms have been identified: cytosolic PGES (cPGES), mPGES-1, and mPGES-2. Cytosolic PGES is constitutively and ubiquitously expressed and is preferentially coupled with COX-1. In contrast, mPGES-1 is up-regulated by proinflammatory stimuli and is functionally coupled with COX-2; mPGES-2 is ubiquitously expressed in diverse tissues, but the role of mPGES-2 remains to be elucidated (34, 35).

Four PGE2 receptor subtypes have been cloned in mice and thoroughly characterized. The EP4 subtype of the PGE2 receptor on osteoblasts is involved in osteoclast formation by LPS, TNF-α, and IL-1 (30). Therefore, PGE2 production caused by increased expression or activity of cPLA2, COX-2, and mPGES-1 in osteoblasts after treatment with LPS or proinflammatory cytokines might increase osteoclast formation through the EP4 subtype on osteoblasts. LPS and proinflammatory cytokines, including IL-1, promote PGE2 production by increasing COX-2 expression in a manner dependent on NF-kB activation (36, 37). NF-kB has been proposed to be a redox-sensitive transcription factor (38), and LA inhibited TNF-α-induced NF-kB by inhibiting the activities of IkB kinase (39). However, in this study LA (20 μM) inhibited PGE2 synthesis by IL-1 even in the presence of arachidonic acid, without affecting the protein expression level of cPLA2, COX-2, and mPGES in osteoblasts (Fig. 6). Thus, it is unlikely that LA (20 μM) affects IL-1-induced signaling pathways, including NF-kB activation, required for the increases in the enzymes involved in PGE2 biosynthesis.

COX, a heme-containing protein, is a bifunctional enzyme exhibiting both COX and peroxidase activities. The COX component converts arachidonic acid to PGG2, and the peroxidase component reduces the endoperoxide to PGH2 (40). Endogenous radicals are required to activate newly made COX holoenzymes by forming tyrosyl radical at Tyr385, which is crucial for the catalytic activity. Lipid peroxides and peroxynitrite have both been implicated as the oxidants that oxidize ferric heme to a ferryl-oxo protoporphyrin radical (40). Previous reports have shown that LA can regenerate endogenous and exogenous antioxidants and scavenge free radicals, including lipid peroxides and peroxynitrite, in cells (13, 41, 42). Thus, this antioxidant property of LA may be involved in the inhibition of COX-2 and the subsequent decrease in PGE2 synthesis. It was also recently reported that DHLA, but not LA or the hydrophilic thiol NAC, inhibits lipid peroxidation by 15-lipoxygenase, a member of the family of nonheme iron-containing dioxygenases (43). This effect was suggested to stem primarily from the reduction of the active ferric 15-lipoxygenase form to the inactive ferrous state after hydrophobic interaction between DHLA and the enzyme and, possibly, from scavenging of fatty acid peroxyl radicals formed during lipoperoxidative processes (43). In our study DHLA, but not LA, decreased the peroxidase activity of human COX-2 (Fig. 6), indicating that the suppression of PGE2 production in LA-treated cells was due to COX-2 inhibition by DHLA generated by reduction of LA in the cells. Interestingly, GSH (100 μM) decreased COX-2 activity by ~70% in vitro, but had only a marginal effect on PGE2 synthesis by IL-1 in osteoblasts even at 1–10 mM (Fig. 6). Given that DHLA, but not GSH, is hydrophobic (17) and that mPGES and both COX isoenzymes are localized in the perinuclear envelope (44), DHLA may be more effective to inhibit COX-2 activity and PGE2 production than GSH in cells. However, hydrophobicity may not be a factor that affects the activities of those thiol oxidants in an in vitro assay system of COX-2. In addition, GSH can function as a cofactor of PGES in vivo (45, 46), which compensates for the inhibition of COX-2 by GSH.

We also found that the i.p. administration of LA could reduce osteoclast formation and bone loss induced by IL-1 in parietal bone (Fig. 7). Recently, it was reported that i.p. pretreatment with LA (50 mg/kg body weight) significantly alleviated LPS-induced tissue injuries by decreasing LPS-induced TNF-α and NO concentrations in plasma and lipid peroxidation in liver (47). In this study daily injection of LA (25 mg/kg) significantly reduced LPS-induced bone loss as well as osteoclast formation, most likely by inhibiting PGE2 production (Fig. 7). Thus, these results suggest that exogenous application of LA reduces bone destruction in inflammatory conditions.

In this study, we showed that LA inhibited PGE2 synthesis by inhibiting COX-2 activity. Our results indicate the possibility that LA could have beneficial effects on preventing several diseases mediated by PGE2 overproduction as well as osteoclastic bone loss associated with inflammation.

Disclosures
The authors have no financial conflict of interest.
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